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ENVIRONMENTALLY INDUCED REVERSIBLE PROTEIN TRANSLOCATION ACROSS INNER MITOCHONDRIAL MEMBRANE

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Four molecular mechanisms account for protein translocation across biological membranes: translocation via vectorial translation, via vectorial processing, via subunit dissociation, and via environmental induction. Whereas the first three mechanisms are vectorial, the last one is reversible (1). Fig. 1 describes the general methodology and observations that led us to the discovery of this last mechanism.

RESULTS AND DISCUSSION

Externalization of a protein such as aspartate aminotransferase (AAT) from the inner face of inner mitochondrial membrane towards intermembranal fluid is shown in Fig. 1, step 1. This step is induced by movement effectors, e.g., succinate, fumarate, malate, pyruvate, citrate, phenylsuccinate, α-methylglutamate, etc., at concentrations ranging from 0 to 50 mM (2). Step 2 shows internalization of previously externalized AAT upon removal of the movement effector. This was demonstrated by testing the accessibility of the enzyme protein first on the outer face of the mitoplast (mitochondria from which the outer membrane was removed), then on the outer face of the corresponding inverted vesicle, which is equivalent to the inner face of the mitoplast (step 3). No permeant substrate, controlled

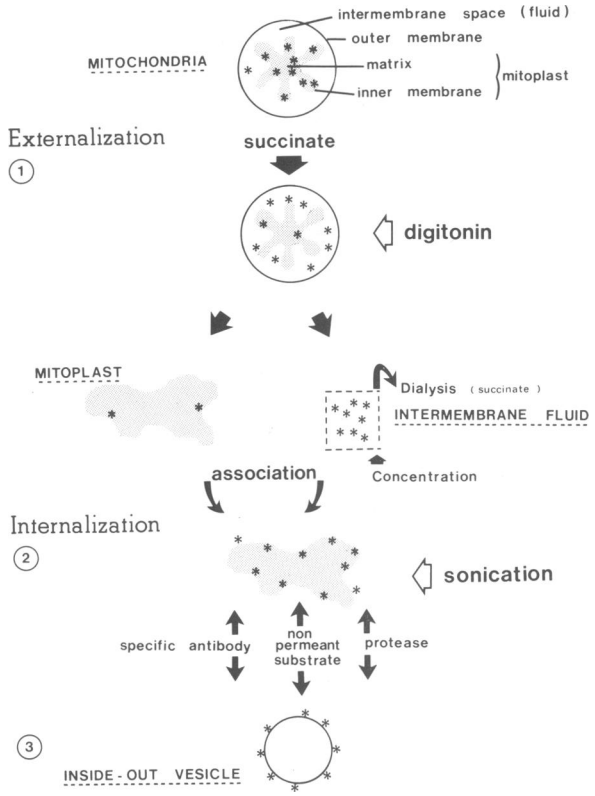

FIGURE 1 Environmentally induced aspartate aminotransferase movement within the mitochondria (10% AAT).

protease digestion, and specific antibody were used as criteria of accessibility. Outer membrane was separated from mitoplast after incubation of 10 mg mitochondrial protein with 1 mg digitonin (3). Specific reorganization of membrane and adjoining subcompartment following exposure to various movement effectors was estimated by showing : (*a*) the specific modification of intermembrane fluid protein patterns provoked by different movement effectors as revealed by polyacrylamide gel electrophoresis (2); and (*b*) the differential localization of AAT in the mitochondrial compartments in the presence of different movement effectors (3) (Table I). These results not only

TABLE I
INFLUENCE OF THE NATURE OF MOVEMENT EFFECTOR ON THE SUBMITOCHONDRIAL LOCALIZATION OF ASPARTATE AMINOTRANSFERASE

Movement effector	Percent of total aspartate aminotransferase activity in different submitochondrial compartments		
	Intermembrane space (fluid)	Inner membrane	Matrix
Sucrose	9	79	12
Phosphate	19	68	13
Acetate	20.5	72	7.5
Succinate	39	25	36
Fumarate	79	6	15
Oxaloacetate	77.5	11.5	11
Citrate	59.5	1.5	39

Results are expressed as percent of total asparate aminotransferase activity. Total aspartate aminotransferase was constant to within ±5%. Sucrose was 0.25 M. Effectors were at 2 μmol/mg of mitochondrial protein.

confirm the specific action of effectors and repartition of membrane and perimembrane protein, but also demonstrate externalization. Inner membrane was separated from matrix after incubation of 1 mg mitoplast protein with 0.16 mg Lubrol WX.

This protein movement is dependent (4) on membrane fluidity and on temperature. The richer the membrane in polyunsaturated fatty acids, the lower the temperature of the break of the Arrhenius curve accounting for the movement effector induced protein externalization. In the case of the two populations of brain mitochondria (cell body and synaptosomal), when the ratio of saturated to polyunsaturated fatty acids varies from 1.07 to 0.62 the temperature of the break of the Arrhenius curve for AAT externalization varies from 27°C to 15°C.

Studies on the temperature-dependent movement of AAT in structurally intact mitochondria were performed by measuring reversible loss of AAT latency in the presence of 1.8 mM succinate using nonpermeant NADH at various temperatures (1, 3).

The results confirmed that AAT-reversible movements

TABLE II
DRIVING FORCE FOR PROTEIN MOVEMENT

Possible nature	Results	Conclusions
Energy-rich intermediate (ATP)	No inhibition of reversible movement by antimycin A, rotenone, oligomycin, 2–4 DNP, amytal, atractylate. Also triggered by phenylsuccinate, and α-methyl glutamate (2)	No energy rich link required
Modification of the primary structure of mobile protein	No modification of molecular weight of the "mobile AAT" as attested by gel electrophoresis on externalized, internalized and reexternalized ^{125}I enzyme*	No modification of primary structure required
Modulation of chemical potential of interacting consitutants	"Spontaneous" internalization of mitochondrial aspartate aminotransferase within negatively charged liposomes as shown by latency of enzyme and inaccessibility to protease digestion (6)	Possible role of the modificatin of chemical potential

*Crémel, G., P. Hubert, and A. Waksman; data to be published.

occur *in situ* and showed that succinate externalization of AAT requires a more fluid membrane than for its internalization. As a matter of fact, internalization still occurs at temperatures below the thermotropic fusion point of the membrane for the externalization of the enzyme.

This asymmetrical recognition of both the externalizing signal and the molecule to be transitorily internalized was also suggested by cross-linking experiments. After treating mitochondria with dimethyladipimidate, externalization of AAT was no longer triggered by succinate, whereas in its absence, internalization of 70% of the enzyme still occurred on cross-linked organelles. This suggests that a greater rigidity or stability of the membrane could be required for the recognition and integration of protein signals as opposed to recognition of ionic signals (5). The driving force of this protein movement seems to be dependent on the chemical potential of both mobile protein and membrane as modulated by environment rather than by hydrolysis of an energy rich bond, or modification of the primary structure of the mobile protein (Table II).

Configurational energy and cognitive properties could thus be responsible for the externalization-internalization phenomenon.

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FORMATION OF THE ENVELOPE OF ROUS SARCOMA VIRUS AND VESICULAR STOMATITIS VIRUS FROM LOCALIZED LIPID REGIONS IN THE PLASMA MEMBRANE

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The phospholipid polar head group composition of chicken embryo fibroblasts and enveloped viruses grown on these cells can be altered by supplementing the culture media with polar head group analogues. Studies of the incorporation of analogues into host cell plasma membranes and virus envelopes have shown that the viruses bud from localized lipid regions which are different from the average composition of the plasma membrane (1). This selective incorporation of phospholipids into the virus envelope may result from a specific requirement of the virus to regulate the envelope membrane structure. To test this hypothesis, the effects of altering the host cell plasma membrane lipid composition and fluidity on the viral envelope lipid composition and fluidity were determined. Measurements were made with 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe of membrane structure and fluidity using steady-state anisotropy methods and differential polarized phase fluorometry (2).

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METHODS

Chicken embryo fibroblasts were cultured as previously described (3). RSV-T5-infected cells growing at 41°C were split into fresh medium, and half were incubated at 41°C and half at 36°C. After 32 h the medium was changed, and viruses and cells were harvested 12 h later. Polar head group modification of phospholipids was done on tertiary cultures using previously described methods (1). Cells were infected for 12 h with VSV (San Juan strain) and viruses and cells harvested after 36 h growth in choline- or *l*-2-amino-1-butanol-supplemented media. Virus purification, plasma membrane isolation, and phospholipid analysis were done as previously described (1). Membranes were labeled with DPH as previously described (4).

RESULTS

RSV-T5 is a Rous sarcoma virus mutant which infects cells and replicates equally well at 36°C and 41°C. At